Use of a HIV-1 Retroviral Vector System for Gene Transfer into Human Cells

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The transfection of HIV 1 proviral DNA and 3'LTR-CAT plasmids by the DEAE dextran method or protoplast fusion was studied previously in H9 cells, from a T4-lymphocyte cell line. The transient expression of different HIV 1 LTR-CAT DNAs was reproducible for all experiments [1]. However, we failed to establish cell lines of nonreplicating HIV 1 proviruses into H9 cells to study viral genes by this method. Here we investigate the selection for a hygromycin-B gene transfected into H9 cells. Hygromycin B, an aminoglycoside antibiotic produced by *Streptomyces hygroscopicus,* was used to select transfected H9 cells.

Retroviral vectors have been constructed for gene transfer in mammalian and avian cells. However, they are restricted in host range. We describe a retroviral vector system based entirely on a human immunodeficiency virus (HIV 1) with the ability to carry out efficient gene transfer into human cells.

By establishing a helper cell line that produces the *trans*-acting viral gene products, we propagate the *cis*-acting components in them and harvest defective viral particles that contain only the *cis*-acting components. The packaging signal in HIV 1 has not been identified. We postulate that the sequence for the packaging signal is located between the primer binding site (PBS) and the gag genes. Therefore, we constructed deletion mutants in this region and transfected these mutants into H9 cells. The plasmid pHU3d containing the complete proviral genome of HIV 1 and a hygromycin-B gene for selection were used for construction. An additional deletion in the 3'LTR (−138 to −48) has been introduced into all plasmids. We established cell lines with the mutants and studied the reverse transcriptase activity and protein synthesis.

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Reference

1. Heisig V, Benter T, Josephs SF, Sadaie MR, Okamoto T, Gallo RC, Wong-Staal F (1987) Interaction of viral and cellular factors with the HTLV-LTR target sequences in vitro. In: Neth R, Gallo RC, Greaves MF, Kabisch H (eds) Modern trends in human leukemia 7. Springer, Berlin Heidelberg New York, p 423